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(54) Recombinant DNA sequences encoding feedback inhibition released enzymes, plasmids comprising the recombinant DNA sequences, transformed microorganisms useful in the production of aromatic amino acids, and a process for preparing aromatic amino acids by fermentation

(57) A method for releasing feedback inhibition of the key enzymes in the production of aromatic amino acids by fermentation is disclosed. Aromatic amino acids are prepared by a process which comprises transforming a microorganism with a recombinant DNA sequence bearing a gene or gene group encoding a feedback inhibition-released enzyme in the phenylalanine and/or tryptophan biosynthetic pathway, obtained by substituting one or two amino acid residue(s) or deleting one or more amino acid residue(s) of 3-deoxy-D-arabinohexulonate 7-phosphate synthase (DS) or prephenate dehydratase, culturing the microorganism and isolating the aromatic amino acid produced in the medium. Higher efficiency and improved yields in the production of L-phenylalanine and L-tryptophan by fermentation are realized.

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Description**BACKGROUND_OF THE INVENTION**5 **Field of the Invention:**

The present invention relates to recombinant DNA sequences encoding feedback inhibition released enzymes, plasmids containing these recombinant DNA sequences, microorganisms transformed with these plasmids, and a process for preparing L-tryptophan, L-phenylalanine and L-tyrosine by fermentation.

10 **Discussion of the Background:**

Demand for aromatic amino acids is rapidly increasing. For example, L-phenylalanine is used as a raw material for the sweetener aspartame, L-tryptophan is an important feed additive, and all three (L-phenylalanine, L-tryptophan, and L-tyrosine) are useful as transfusion drugs.

15 Many methods for preparing aromatic amino acids using microorganisms are known. For example, methods for preparing L-phenylalanine using recombinant *Escherichia coli* are described in Japanese Published Unexamined Patent Application Nos. 56-1890, 58-103398, 61-92565 and 1-104160, and World Patent Publication WO 87/00202. A method for preparing L-phenylalanine or L-tyrosine using a mutant belonging to *Coryneform* bacteria is described in Japanese Patent Published Unexamined Application No. 61-128897, and methods using recombinant *Coryneform* bacteria are described in Japanese Unexamined Published Patent Application Nos. 60-34197, 60-24192, 61-260892 and 61-124375. A method for preparing L-tryptophan using recombinant *E. coli* is described in Japanese Published Unexamined Patent Application No. 57-71397 and U.S. Patent No. 4,371,614; methods using mutants of *Bacillus subtilis* are described in Japanese Published Unexamined Patent Application Nos. 53-39517 and 62-34399; methods using recombinant *Bacillus subtilis* are described in Japanese Patent Published Unexamined Application Nos. 61-104790 and 62-34399; methods using a mutant of *Coryneform* bacteria are described in Japanese Published Unexamined Patent Application No. 57-174096; and a method using recombinant *Coryneform* bacteria is described in Japanese Published Unexamined Patent Application No. 62-51980.

20 Generally, in the biosynthetic route of aromatic amino acids, a key enzyme which plays a central role in the biosynthesis is subject to feedback inhibition by the final product. In the methods described above, the desired amino acids are principally produced using microorganisms wherein the key enzyme is released from feedback inhibition by the final product. The key enzymes released from feedback inhibition in the above methods include 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase (hereafter abbreviated as "DS") and prephenate dehydratase (hereafter abbreviated as "PD").

25 Turning first to DS, among the microorganisms used in the methods described above, *Escherichia coli* has three types of naturally-occurring (wild-type) DS isozymes. These isozymes are encoded by genes called *aroF*, *aroG* and *aroH*, which are subject to feedback inhibition by L-tyrosine, L-phenylalanine and L-tryptophan, respectively.

30 The nucleotide sequences and amino acid sequences relevant to these genes and enzymes are already reported [*aroF*: Hudson, G.S. and Davidson, B.E., *J. Mol. Biol.*, **180**, 1023 (1984); *aroG*: Davies, W.D. and Davidson, B.E., *Nucleic Acids Res.*, **13**, 4045 (1982); *aroH*: Ray, J.M. et al., *J. Bacteriol.*, **170**, 5500 (1988)].

35 In order to efficiently produce the desired aromatic amino acids, expression of these DS genes must be improved. With respect to *aroH*-encoded DS, feedback inhibition by L-tryptophan has been released using mutant *aroH* [Ray, J.M. et al., *J. Bacteriol.*, **170**, 5500 (1988)]. However, the DS activity derived from *aroH* is very poor, and the *aroH*-derived DS is unsuitable for improvement by recombinant DNA techniques. It is more efficient to utilize *aroF*- or *aroG*-encoded DS in which feedback inhibition is released ("feedback inhibition-released" DS).

40 An example of a mutation which releases feedback inhibition of *aroE*-encoded DS by L-tyrosine is the substitution of the 148 proline residue from the N-terminus (¹⁴⁸Pro) with a leucine residue [Weaver, L.M. and Herrmann, K.M., *J. Bacteriol.*, **172**, 6581 (1980)].

45 Only a few examples as shown below for the production of aromatic amino acids by fermentation employ feedback inhibition-released DS with a clearly shown mutation site. Edwards et al. teach that feedback inhibition by L-tyrosine in the production of L-phenylalanine by fermentation is released by substituting the 152 glutamine residue (¹⁵²Gln) of DS encoded by *aroE* with isoleucine [WO 87/00202]. Furthermore, Sinenki et al. teach that feedback inhibition by L-phenylalanine in the production of L-phenylalanine by fermentation is suppressed by substituting the 76 leucine residue (⁷⁶Leu) of DS encoded by *aroG* with valine [Japanese Published Unexamined Patent Application No. 58-103398]. However, the enzyme activity of the feedback inhibition-released DS and the amount of L-phenylalanine produced are unknown. No reports of the production of L-tryptophan by feedback inhibition-released DS mutants are known.

50 Turning next to PD, a wild-type bifunctional enzyme (CM-PD) present in *Escherichia coli* having both chorismate mutase (hereafter abbreviated as "CM") activity and PD activity is subject to feedback inhibition by L-phenylalanine. The enzyme is encoded by a gene called *pheA*. The nucleotide sequence of *pheA* and the amino acid sequence of wild-type

CM-PD are known [Hudson, G.S. and Davidson, B.E., J. Mol. Biol., 180, 1023 (1984)]. In order to efficiently produce L-phenylalanine, it is important to release the feedback inhibition of CM-PD by L-phenylalanine.

Some examples of modification and mutation on an amino acid level are known to release feedback inhibition for the fermentative production of L-phenylalanine. By modifying two tryptophane residues (226 and 338 amino acids from the N-terminus) of CM-PD with dimethyl(2-hydroxy-5-nitrobenzylsulfonium bromide), an enzyme having resistance to feedback inhibition can be obtained [Gething, M.J.H. and Davidson, B.E., Eur. J. Biochem., 78, 111 (1977)]. Feedback inhibition-released enzyme can be obtained by deleting the 338 tryptophan residue (³³⁸Trp) or substituting ³³⁸Trp and the subsequent residues with arginine-glycine (Japanese Published Unexamined Patent Application No. 1-235597). Inserting the amino acid sequence tryptophan-arginine-serine-proline into the site of the same 338 tryptophan residue also releases feedback inhibition (WO 87/00202). These techniques focus on the 338 tryptophan residue. However, no definitive study on the effects of modifying or mutating the ²²⁶Trp residue has been performed.

On the other hand, in Coryneform bacteria, PD is subject to feedback inhibition by L-phenylalanine. A gene in which the feedback inhibition by L-phenylalanine has been released is known. [Ozaki, A. et al., Agric. Biol. Chem., 49, 2925 (1986); Ito, H. et al., Appl. Microbiol. Biotechnol., 33, 190 (1989)]. The nucleotide sequence of the wild type Coryneform PD gene shows homology to the pheA gene of Escherichia coli K-12 [Follettie, M.T. and Sinsky, A.J., J. Bacteriol., 167, 695 (1986)]. However, the nucleotide sequence of the feedback inhibition-released PD gene in Coryneform bacteria is unknown, as is mutation of the nucleotide sequence and release of feedback inhibition by substitution of the corresponding amino acid sequence.

20 SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide a process for efficiently preparing an aromatic amino acids by fermentation.

A further object is to provide transformed microorganisms useful in the production of aromatic amino acids by fermentation.

A further object is to provide recombinant plasmids which express genes encoding key enzymes in the biosynthesis of aromatic amino acids in which feedback inhibition is released.

A further object is to provide recombinant DNA sequences which encode key enzymes in the biosynthesis of aromatic amino acids in which feedback inhibition is released.

A further object is to provide novel recombinant enzymes which are important in the biosynthesis of aromatic amino acids in which feedback inhibition is released.

These and other objects which will become apparent during the following detailed description of the preferred embodiments have been accomplished by a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released, a plasmid comprising the recombinant DNA sequence, a microorganism useful in the production of aromatic amino acids transformed with one or more of the plasmids, and a process for preparing an aromatic amino acid which comprises culturing the transformed microorganism and isolating the aromatic amino acid produced thereby.

40 BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

- 45 Fig. 1 shows the construction of the plasmids pTS-aroF and pTS-aroG;
- Fig. 2 shows the extent of inhibition by L-tyrosine of activity in DS encoded by both wild-type and mutant aroF;
- Fig. 3 shows the extent of inhibition by L-phenylalanine of activity in DS encoded by both wild type and mutant aroG.
- Fig. 4 shows the extent of inhibition by L-phenylalanine in the prephenate dehydratase activity of both wild-type and mutant chorismate mutase-prephenate dehydratase;
- 50 Fig. 5 shows the construction of the plasmid pACKG4.

55 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

- The present invention relates to a mutant feedback inhibition-released enzyme in the biosynthetic pathway of aromatic amino acids; a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released; a plasmid comprising a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released; a microorganism useful in the production of aromatic amino acids transformed with one or more plasmids comprising a recombinant DNA sequence

encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released; and a process for preparing an aromatic amino acid which comprises culturing a microorganism transformed with one or more plasmids comprising a recombinant DNA sequence encoding an enzyme of the aromatic amino acid biosynthetic pathway, wherein feedback inhibition is released, and isolating the aromatic amino acid produced thereby.

5 In the present application, the phrase "aromatic amino acid" refers to L-phenylalanine, L-tryptophan and L-tyrosine. Also, an enzyme is "released" from feedback inhibition by a final product if the activity of the enzyme doesn't decrease in the presence of the final product.

10 Preferably, the enzymes of the present invention in the biosynthetic pathway of aromatic amino acids which are to be released from feedback inhibition are 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase (DS), prephenate dehydratase (PD) and chorismate mutase-prephenate dehydratase (CM-PD). The means by which each of these enzymes is released from feedback inhibition in the present invention is preferably by mutation, wherein one or two amino acid residue(s) are substituted with other amino acid residue(s) or one or more amino acid residue(s) are deleted. Furthermore, the transformed microorganism preferably belongs to the genus Escherichia, and is preferably transformed with one or more plasmids bearing a recombinant DNA sequence corresponding to one of the above 15 mutant enzymes.

20 Firstly, the present Inventors have acquired a novel gene encoding DS, wherein the feedback inhibition is released by cloning the natural DS gene of Escherichia coli and subjecting the cloned gene to mutation. Furthermore, the natural PD gene of Brevibacterium lactofermentum is cloned, and a gene encoding PD in which feedback inhibition is released, is cloned from L-phenylalanine-producing Coryneform bacteria. Even further, the natural CM-PD gene of Escherichia coli is cloned, and the cloned gene is then subjected to mutation to produce a novel gene encoding CM-PD in which feedback inhibition is released. By transfecting or transforming phenylalanine-producing bacteria with one or two of the genes of the present invention, production of L-phenylalanine by fermentation can be improved.

25 The present Inventors have also improved the fermentative production of L-tryptophan by transforming a microorganism with the novel DS gene of the present invention in combination with a tryptophan operon, in which the feedback inhibition of anthranilate synthase (hereafter abbreviated as AS), an enzyme for the L-tryptophan biosynthesis system, is also released.

The novel gene of the present invention encoding DS having released feedback inhibition is prepared by the following procedure.

30 Firstly, aroF and aroG genes are cloned from chromosomal DNA of Escherichia coli using the PCR method as described in U.S. Patent Nos. 4,800,159, 4,683,202 and 4,683,195, all incorporated herein by reference. The chromosomal DNA appropriate as a source of the aroF and aroG genes for use in the present invention may be cloned from any strain of Escherichia coli, but the preferred strain is K-12 MC1061 (ATCC 5338). The desired genes are then mutated with hydroxylamine by a known method; for example, that described in J. Mol. Biol., 175, 331 (1984).

35 The genes aroE and aroG encode DS which is subject to feedback inhibition by L-tyrosine and L-phenylalanine, respectively, and also include mutants caused by genetic polymorphism, etc. Genetic polymorphism refers to a modification of an amino acid sequence of a protein due to natural mutation of a gene.

40 In order to cause mutation of the gene, a number of effective methods are known. Examples include recombinant PCR methods [PCR Technology, Stockton Press (1989)], site specific mutation [Kramer, W. and Frits, H.J., Methods in Enzymology, 154, 350 (1987)], conventional methods exposing a strain bearing the gene to UV (ultraviolet light) rays, conventional methods treating the DNA or DNA-bearing microorganism with a chemical (N-methyl-N'-nitrosoguanidine, nitric acid, etc.), and conventional methods for chemical synthesis of the desired gene, such as those employing a known automated synthesizer.

45 In the present invention, the mutated amino acid residue of DS is in the region of the amino acid sequence which participates in the mechanism of feedback inhibition by L-tyrosine, L-phenylalanine or L-tryptophan. For example, in DS encoded by aroF, the 147 aspartic acid residue (¹⁴⁷Asp) and the 181 serine residue (¹⁸¹Ser) from the N-terminus are the mutated amino acid residues. Any mutation of the amino acid residue which results in release from feedback inhibition is suitable. For example, substitution, deletion, or addition is suitable. The DS mutations and the corresponding nucleotide sequence mutations exemplified in the present invention are summarized in Table 1.

50 By transfecting a suitable microorganism with the mutant aroF or aroG gene above as a recombinant DNA sequence, the microorganism can express the recombinant mutant gene in which feedback inhibition is released.

In the present invention, the novel gene encoding PD in Brevibacterium lactofermentum and the novel gene encoding CM-PD in Escherichia coli were prepared as follows.

55 Firstly, the nucleotide sequence of the Brevibacterium lactofermentum PD gene encoding PD in which feedback inhibition by L-phenylalanine is released was determined and analyzed. It has thus been found that the L-phenylalanine-producing strain expressed PD in which one amino acid is substituted, as compared to the wild strain. Next, based on this finding, a substitution or a deletion of amino acid residue(s) was carried out at the corresponding position of CM-PD in Escherichia coli K-12, resulting in CM-PD in which the feedback inhibition is released.

Enzymes having PD activity referred to in the present invention refer to enzymes derived from microorganisms such as Coryneform bacteria having PD activity, and further refer to enzymes derived from microorganisms such as

Escherichia coli, etc., having the bifunctional activity of CM-PD.

In the present invention, the mutated amino acid residue of PD refers to a substitution of an amino acid residue or a deletion of amino acid residue(s) present in the region of the amino acid sequence which participates in the mechanism of feedback inhibition by L-phenylalanine. For example, in PD derived from Brevibacterium lactofermentum, the 235 serine residue (²³⁵Ser) is suitable for mutation, and in CM-PD derived from Escherichia coli, the 330 serine residue (³³⁰Ser) from the N-terminus is an amino acid residue suitable for mutation. Suitable mutations include any which result in the release of feedback inhibition, but particularly suitable mutations include substitutions of ²³⁵Ser or ³³⁰Ser with proline or aspartic acid residue, or deletion of amino acid residues down stream from ³³⁰Ser.

5 10 By transfecting a suitable microorganism with the mutant PD or CM-PD gene described above as a recombinant DNA sequence, expression of PD in which feedback inhibition is released is achieved in the transfected microorganism.

The recombinant DNA sequences obtained by the foregoing procedures refer to those obtained by incorporating a gene encoding feedback inhibition-released DS or PD into a vector of plasmid or phage DNA. In the present invention, promoters such as lac, trp, PL, etc. which act in the microorganism may also be used to efficiently perform the expression of the gene. The recombinant DNA sequences referred to herein include those obtained by incorporating one or more of the above-described genes into a chromosome according to known methods. Examples include methods using a transposon (Berg, D.E. and Berg, C.M., Bio/Technol., 1, 417 (1983)), Mu phage (Japanese Published Unexamined Patent Application No. 2-109985) or homologous recombination [Experiments in Molecular Genetics, Cold Spring Harbor Laboratory (1972)].

15 20 As the microorganism containing the recombinant DNA, any microorganism may be used, irrespective of species and strain of the microorganism, so long as it expresses the gene encoding the desired enzyme (such as DS or PD) which has acquired L-phenylalanine productivity by imparting L-phenylalanine analog resistance thereto). Particularly suitable microorganisms are selected from the genus Escherichia, the genus Brevibacterium, the genus Corynebacterium, the genus Bacillus, the genus Serratia, the genus Pseudomonas, etc.

25 25 The thus obtained microorganism transformed by the recombinant DNA bearing the feedback inhibition-released DS or PD gene is cultured, the desired aromatic amino acid is produced by the transformed microorganism in a suitable medium, and the accumulated aromatic amino acid is collected and isolated.

The medium used for producing the aromatic amino acid is a conventional medium containing appropriate carbon sources, nitrogen sources, inorganic ions and, if necessary, other organic components.

30 Suitable carbon sources include sugars such as glucose, lactose, galactose, fructose, starch hydrolysate, etc.; alcohols such as glycerol, sorbitol, etc.; organic acids such as fumaric acid, citric acid, succinic acid, etc.

Suitable nitrogen sources include inorganic ammonium salts such as ammonium sulfate, ammonium chloride, ammonium phosphate, etc.; organic nitrogen such as soybean hydrolysate, etc.; ammonia gas, ammonia water, etc.

35 Suitable organic trace nutrient sources preferably are present, and include required substances such as vitamin B₁, L-tyrosine, or yeast extract, etc., in an appropriate amount.

In addition thereto, small amounts of potassium phosphate, magnesium sulfate, iron ions, manganese ions, etc. may be present.

40 Incubation is carried out for 16 to 72 hours under aerobic conditions. The temperature for incubation is maintained between 30 and 45°C and the pH is maintained in the range of 5 to 7 during the incubation. The pH may be adjusted with either acids or alkaline substances, which may be inorganic or organic, or may be adjusted with ammonia gas, etc., as is appropriate to maintain the desired pH and concentrations of components in the medium.

The desired aromatic amino acid is isolated from the fermentation medium generally by conventional methods, such as use of an appropriate ion exchange resin, precipitation, and/or other known techniques, either alone or in combination.

45 45 By the general process described above, the transformant expressing feedback inhibition-released DS, PD and/or CM-PD is obtained, and by culturing the transformant, the productivity of aromatic amino acids can be greatly improved.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention, and are not intended to be limiting thereof.

50 50 Example 1: Preparation of a novel gene encoding DS in which the feedback inhibition is released

(1) Collection of an aroF-derived mutant DS gene of Escherichia coli

55 Chromosomal DNA was extracted from Escherichia coli K-12 MC1061 strain in a conventional manner. In a separation procedure, two synthetic DNA primers shown by Sequence Nos. 1 (SEQ ID NO:1) and 2 (SEQ ID NO:2) were synthesized in a conventional manner, based on the known nucleotide sequence of the target aroF gene [J. Mol. Biol., 180, 1023 (1984)].

Sequence No. 1 GCTAACCAAGT AAAGCCAACA (SEQ ID NO:1)

Sequence No. 2 CCCACTTCAG CAACCAGTTC (SEQ ID NO:2)

These primers have homologous sequences upstream and downstream from the aroF gene. Using the chromosomal DNA and the DNA primers, PCR (polymerase chain reaction) is conducted according to the method of Erlich et al. [PCR Technology, Stockton Press (1989)], yielding a DNA fragment of 1.5 Kbp. Thereafter, as shown in Fig. 1, left side, the fragment is cleaved with restriction enzymes EcoRI and Eco47III, and the product is then ligated with the SmaI digestion product of pHSG398 (manufactured by Takara Shuzo) using T4 DNA ligase. Competent cells of Escherichia coli JM109 strain (manufactured by Takara Shuzo) were transformed with the reaction mixture. A plasmid having the aroF gene was extracted from the strains resistant to chloramphenicol to yield the plasmid pHSG-aroF.

Subsequently, pHSG-aroF was digested with restriction enzymes EcoRI and HindIII, and the resulting DNA fragment bearing the aroF gene was ligated with the EcoRI and HindIII digestion fragment of plasmid pTS1 (Japanese Patent Application No. 2-192162) using T4 DNA ligase. Competent cells of DS-deleted (aroF, aroG, aroH) strain AB3257 of Escherichia coli K-12 were transformed with the reaction mixture (AB3257 strain was acquired from the Escherichia coli Genetic Stock Center). From among the strains resistant to ampicillin, the strain in which auxotrophy of L-tyrosine, L-phenylalanine and L-tryptophan disappeared was selected, and a plasmid was extracted therefrom, yielding plasmid pTS-aroF.

Next, after mutation of plasmid pTS-aroF using hydroxylamine according to the method of J. Mol. Biol., 175, 331 (1984), the mutant was used to transform the E. coli AB3257 strain. After ampicillin-resistant strains were collected, two strains which grew in minimum medium supplemented with 1 mM L-tyrosine were selected. From these strains, plasmids pTS-aroF15 and pTS-aroF33 bearing the genes encoding feedback inhibition-released DS were obtained.

Cells of AB3257 strain transformed with plasmids containing the gene encoding non-feedback inhibition-released DS are subject to feedback inhibition at 1 mM concentration of L-tyrosine in the minimum medium. Accordingly, the strain subject to feedback inhibition failed to synthesize aromatic amino acids such as L-phenylalanine or L-tryptophan, and failed to grow.

25 (2) Preparation of aroG-derived mutant DS gene of Escherichia coli

A mutant aroG gene was collected in a manner similar to the case of the aroF gene. Two synthetic DNA primers shown by Sequence Nos. 3 (SEQ ID NO:3) and 4 (SEQ ID NO:4) were synthesized in a conventional manner, based on the known nucleotide sequence of the aroG gene (Nucleic Acids Res., 10, 4045 (1982)).

Sequence No. 3 GTATTACCC CGTTATTGTC (SEQ ID NO:3)

Sequence No. 4 ACTCCGCCGG AAGTGACTAA (SEQ ID NO:4)

35 Using the primers and the chromosomal DNA of the E. coli MC1061 strain, PCR was carried out to obtain a DNA fragment of 2.1 Kbp. As shown in Fig. 1, right side, the fragment was cleaved with restriction enzymes Sall and Eco47III, and the product was then ligated with the Sall and SmaI digestion product of pHSG398 (manufactured by Takara Shuzo) using T4 DNA ligase. Competent cells of Escherichia coli JM109 strain were transformed with the reaction mixture. From among the strains resistant to chloramphenicol, a plasmid having the aroG gene was extracted to yield the plasmid pHSG-aroG.

40 Subsequently, pHSG-aroG was digested with restriction enzymes EcoRI and HindIII, and the resulting DNA fragment bearing the aroG gene was ligated with the EcoRI and HindIII digestion fragment of plasmid pTS1 using T4 DNA ligase. From among the grown strains resistant to ampicillin, the strain in which auxotrophy of L-tyrosine, L-phenylalanine and L-tryptophan disappeared was selected, and a plasmid was extracted therefrom to yield the plasmid pTS-aroG.

45 Next, after mutation of the plasmid using the hydroxylamine method of Example 1-(1) above, the mutant plasmid was used to transform competent cells of the E. coli AB3257 strain. After ampicillin-resistant strains were isolated, 6 strains which grew in minimum medium supplemented with 10 mM L-phenylalanine were selected. From these strains, plasmids pTS-aroG4, pTS-aroG8, pTS-aroG15, pTS-aroG17, pTS-aroG29 and pTS-aroG40 bearing the aroG gene encoding feedback inhibition-released DS were obtained.

In cells of the AB3257 strain expressing the non-feedback inhibition-released DS, feedback inhibition occurs at a concentration of 10 mM L-phenylalanine in minimum medium. Accordingly, the non-feedback inhibition-suppressed strain fails to synthesize aromatic amino acids such as L-tryptophan and/or L-tyrosine, and therefore, fails to grow.

55 (3) Determination of DS enzyme activity

The above plasmids, bearing either mutant aroF (pTS-aroF15 and pTS-aroF33) or mutant aroG (pTS-aroG4, pTS-aroG8, pTS-aroG15, pTS-aroG17, pTS-aroG29 and pTS-aroG40), were used to transform Escherichia coli AB3257 strain having no DS activity. The respective transformants were named AJ 12598 (AB3257/pTS-aroF15), AJ 12599

(AB3257/pTS-aroF33), AJ 12562 (AB3257/pTS-aroG4), AJ 12600 (AB3257/pTS-aroG8), AJ 12563 (AB3257/pTS-aroG15), AJ 12601 (AB3257/pTS-aroG17), AJ 12602 (AB3257/pTS-aroG29) and AJ 12603 (AB3257/pTS-aroG40). Among them, AJ 12563 and AJ 12603 were deposited as representative strains in the Fermentation Research Institute of the Agency of Industrial Science & Technology of Japan, under the deposit numbers Escherichia coli FERM BP-3567 and FERM BP-3568, respectively. For the purpose of comparison, plasmids bearing wild type genes were also used to transform the E. coli AB3257 strain.

Each of these strains were cultured for 24 hours in a known L-phenylalanine-producing medium [Sugimoto, S. et al., J. Biotechnol., 5, 237 (1988)]. From the culture cells, the crude enzyme solution was prepared by ultrasonic homogenization. The enzyme activity of DS was determined in a conventional manner [Gollub, E. et al., Methods Enzymol., 17, 349], in the presence of L-tyrosine in the case of aroE and in the presence of L-phenylalanine in the case of aroG. The results presented in Figs. 2 and 3 show that the DS enzyme activity of the wild type transformants (Escherichia coli AB3257/pTS-aroF) is strongly inhibited in the presence of L-tyrosine, whereas the respective mutant transformants released from feedback inhibition by L-tyrosine. Likewise, in the wild type transformant Escherichia coli AB3257/pTS-aroG, the enzyme activity is strongly inhibited in the presence of L-phenylalanine, whereas in the respective mutant transformants, feedback inhibition by L-phenylalanine is released. Furthermore, the mutant strain AJ 12562 not only of L-phenylalanine increases.

(4) Determination of the mutation site of DS in which the feedback inhibition is released

The nucleotide sequences of the feedback inhibition-released aroF15, aroF33, aroG4, aroG8, aroG15, aroG17, aroG29 and aroG40 were determined in a conventional manner [Molecular Cloning (Second Edition), Cold Spring Harbor Press (1989)]. The specific substitution site on the amino acid sequence and the mutation site on the corresponding nucleotide sequence are shown in Table 1.

These sequences are all novel.

Table 1

| Mutant Gene | Substitution Site of Amino Acid | | Corresponding Nucleotide Sequence Change |
|-------------|---------------------------------|----------------------------|--|
| | Position from N-Terminus | Amino Acid Sequence Change | |
| aroF15 | 147 | Asp → Asn | GAT → AAT |
| aroF33 | 181 | Ser → Phe | TCC → TTC |
| aroG4 | 150 | Pro → Leu | CCA → CTA |
| aroG8 | 202 | Ala → Thr | GCC → ACC |
| aroG15 | 146 | Asp → Asn | GAT → AAT |
| aroG17 | 147 | Met → Ile | ATG → ATA |
| | 332 | Glu → Lys | GAA → AAA |
| aroG29 | 147 | Met → Ile | ATG → ATA |
| aroG40 | 157 | Met → Ile | ATG → ATA |
| | 219 | Ala → Thr | GCG → ACG |

Example 2: Preparation of a novel gene encoding PD in which the feedback inhibition is released

(1) Determination of the mutation site of Brevibacterium lactofermentum mutant PD

The nucleotide sequence of the NcoI fragment in plasmid pAJ16 bearing the PD gene of Brevibacterium lactofermentum wild strain was determined by the dideoxy method, using the homology to known Corynebacterium s.p. PD gene [Follettie, M.T. and Sinsky, A.J., J. Bacteriol., 167, 695 (1986)] as an index. The plasmid is harbored on Brevibacterium lactofermentum AJ 12125 (FERM P-7546). The resulting nucleotide sequence (SEQ ID NO:5) and the corresponding amino acid sequence (SEQ ID NO:6) are shown below. The B. lactofermentum PD amino acid sequence is

different by only one amino acid residue from that of Corynebacterium s.p.

Sequence 5:

5 ATGAGCGACCCACCAATTGTTGTGGCCTATTCGGGCCTGCCGGAACCTTCACCGAAGAA
MetSerAspAlaProIleValValAlaTyrLeuGlyProAlaGlyThrPheThrGluGlu
1 20

10 GCCCTCTACAAATTGCCGACGCCGGCGTATTCGGCGACGGTGAGATCGAGCAGCTACCA
AlaLeuTyrLysPheAlaAspAlaGlyValPheGlyAspGlyGluIleGluGlnLeuPro
21 40

15 GCCAAATGCCACAAGAAGCTGTCGACGCCGGTCCGCCACGGCACCGCCCAGTCGGTGC
AlaLysSerProGlnGluAlaValAspAlaValArgHisGlyThrAlaGlnPheAlaVal
41 60

20 GTGCCCATCGAAAACCTCGTCGACGGCCCCGTCACCCCCACCTCGACGCCCTGACCAG
ValAlaIleGluAshPheValAspGlyProValThrProThrPheAspAlaLeuAspGln
61 80

25 GGCTCCAACGTGCAAATCATGCCGAAGAAGAACTCGATATTGCCCTTTCCATCATGGTC
GlySerAsnValGlnIleIleAlaGluGluLeuAspIleAlaPheSerIleMetVal
81 100

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5

CGGCCAGGGACTTCGCTTGCACGTCAAACCCCTGCCACCCACCCGGTTGGGTACCAA
ArgProGlyThrSerLeuAlaAspValLysThrLeuAlaThrHisProValGlyTyrGln
101 120

10 CAAGTGAAAAACTGGATGGCAACCACCATTCCGGACGCCATGTATCTTCAGCAAGCTCC
GlnValLysAsnTrpMetAlaThrThrIleProAspAlaMetTyrLeuSerAlaSerSer
121 140

15 AACGGCGCCGCCACAAATGGTGTGCCGAAGGAACGCCGACGCAGCCGAGCGCCCTCC
AsnGlyAlaGlyAlaGlnMetValAlaGluGlyThrAlaAspAlaAlaAlaProSer
141 160

20 CGCGCAGCCGAACCTTCGGACTGGAACGCCCTGTGATGATGTCGCCGACGTCCGCC
ArgAlaAlaGluLeuPheGlyLeuGluArgLeuValAspAspValAlaAspValArgGly
161 180

25 GCCCCCACCGCTTCGTTGCAGTCCAAGCCAAGCAGCCGTTCCGAACCGACCGGCCAC
AlaArgThrArgPheValAlaValGlnAlaGlnAlaAlaValSerGluProThrGlyHis
181 200

30 GACCGCACCTCCGTCACTTCCTACCGAATGTGCCAGGCAGCCTCGTGCAGCCCTC
AspArgThrSerValIlePheSerLeuProAsnValProGlySerLeuValArgAlaLeu
201 220

35 AACGAATTTCGCCATCCGTGGCGTCGACCTCACCGCATCGAATCCGCCAACCGCAAA
AsnGluPheAlaIleArgGlyValAspLeuThrArgIleGluSerArgProThrArgLys
221 240

40 GTCTTCGGAACCTACCGCTTCCACCTGGACATACTGGACATATCCGACATCCCGTC
ValPheGlyThrTyrArgPheHisLeuAspIleSerGlyHisIleArgAspIleProVal
241 260

45 GCCGAAGCCCTCCGCCACTCCACCTCCAAGCCGAAGAACTCGTATTCGTCGGTTCCGG
AlaGluAlaLeuArgAlaLeuHisLeuGlnAlaGluGluLeuValPheValGlySerTrp
261 280

50 CCCTCCAACCGTGCAGAAGACAGCACGCCAAACCGACCAACTAGCTAACGTACACAAG
ProSerAsnArgAlaGluAspSerThrProGlnThrAspGlnLeuAlaAsnValHisLys
281 300

55 CGGGACGAATGGGTTCGCGCAGCAAGCGAAGGAAGGAAACTTAACCTAG
AlaAspGluTrpValArgAlaAlaSerGluGlyArgLysLeuAsn***
301 315

45 Next, the nucleotide sequence (SEQ ID NO: 7) of the gene on plasmid pPH14 encoding PD of the phenylalanine-producing strain of Brevibacterium lactofermentum was determined. The sequence shown below was obtained. The plasmid used was the one borne on Brevibacterium lactofermentum AJ 12259 (FERM BP-3565). A comparison of the amino acid sequences was made between the wild-type PD and the feedback inhibition-released PD (SEQ ID NO: 8) and it was found that ²³⁵Ser residue of the wild strain was mutated to a proline residue in the feedback inhibition-released PD.

Sequence of Brevibacterium lactofermentum PD (pPH14) :

5 ATGAGCGACGCCACCAATTGGTGTGGCCTATTGGGGCCTGCCGGAACCTTCACCGAAGAA
 MetSerAspAlaProIleValValAlaTyrLeuGlyProAlaGlyThrPheThrGluGlu
 1 20
 10 GCCCTCTACAAATTGCGACGCCGGCGTATTGGCGACGGTGAGATCGAGCAGCTACCA
 AlaLeuTyrLysPheAlaAspAlaGlyValPheGlyAspGlyGluIleGluGlnLeuPro
 21 40
 15 GCAAATGCCACAAGAACGCTGTCGACGCCGTCGCCACGGCACCGCCCAGTCGCCGTG
 AlaLysSerProGlnGluAlaValAspAlaValArgHisGlyThrAlaGlnPheAlaVal
 41 60
 20 GTGCCATCGAAAATTCGTCGACGGCCCGTCACCCCCACCTTCGACGCCCTTGACCAAG
 ValAlaIleGluAsnPheValAspGlyProValThrProThrPheAspAlaLeuAspGln
 61 80
 25 GGCTCCAACGTGCAAATCATGCCAGAACGAAACTCGATATTGCCCTTTCCATCATGGTC
 GlySerAsnValGlnIleIleAlaGluGluGluLeuAspIleAlaPheSerIleMetVal
 81 100
 30 CGGCCAGGGACTTCGCTTGCGACGTCAAAACCCCTGCCACCCACCGGTTGGTACCAA
 ArgProGlyThrSerLeuAlaAspValLysThrLeuAlaThrHisProValGlyTyrGln
 101 120
 35 CAACTGAAAAACTGGATGGCAACCACCATTCGGACGCCATGTATTTCAGCAAGCTCC
 GlnValLysAsnTrpMetAlaThrThrIleProAspAlaMetTyrLeuSerAlaSerSer
 121 140
 40 AACGGCGCCGGCGCACAAATGGTTGCCGAAGGAACCGCCGACGCAGCCGAGCGCCCTCC
 AsnGlyAlaGlyAlaGlnMetValAlaGluGlyThrAlaAspAlaAlaAlaProSer
 141 160
 45 CGCGCAGCCGAACCTTCGGAACGCCCTGTTGATGATGTCGCCGACGTCCGCCGC
 ArgAlaAlaGluLeuPheGlyLeuGluArgLeuValAspAspValAlaAspValArgGly
 161 180

50

55

5 GCCCGCACCCGCTTCGTTGCAGTCCAAGCCCCAACGGCAGCGTTCCGAACCGACC GCCAC
 AlaArgThrArgPheValAlaValGlnAlaGlnAlaAlaValSerGluProThrGlyHis
 181
 200
 GACCGCACCTCCGTCACTTCTCCCTACCGAATGTGCCAGGCAGCCTCGCGCGCCCTC
 AspArgThrSerValIlePheSerLeuProAsnValProGlySerLeuValArgAlaLeu
 201
 220
 AACGAATTGCCATCCGTGGCGTCGACCTCACCCGCATCGAAATCCC GCCCCACCCGCAA
 AsnGluPheAlaIleArgGlyValAspLeuThrArgIleGluProArgProThrArgLys
 221
 240
 15 GTCTTCGGAACCTACCGCTTCCACCTGGACATATCCGGACATATCCGCGACATCCCCGTC
 ValPheGlyThrTyrArgPheHisLeuAspIleSerGlyHisIleArgAspIleProVal
 241
 260
 20 GCCGAAGCCCTCCGCGACTCCACCTCCAAGCCGAAGAACTCGTATT CGTCGGTTCCCTGG
 AlaGluAlaLeuArgAlaLeuHisLeuGlnAlaGluGluLeuValPheValGlySerTrp
 261
 280
 25 CCCTCCAACCGTGCAGAAGACAGCACGCCCAAACCGACCAACTAGCTAACGTACACAAG
 ProSerAsnArgAlaGluAspSerThrProGlnThrAspGlnLeuAlaAsnValHisLys
 281
 300
 30 GCCGACGAATGGGTTCGCGCAGCAAGCGAAGGAAGGAAACTTAAC TAG
 AlaAspGluTrpValArgAlaAlaSerGluGlyArgLysLeuAsn***
 301
 315

35 (2) Construction of a novel gene encoding a mutant CM-PD of Escherichia coli

Chromosomal DNA was extracted from Escherichia coli K-12 RRI strain in a conventional manner.
 In a separate procedure, four synthetic DNA primers (Sequence Nos. 7-10) (SEQ ID NOS: 9-12) were chemically synthesized in a conventional manner, based on the known nucleotide sequence of the pheA gene [Hudson, G.S. and Davidson, B.E., J. Mol. Biol., 180, 1023 (1984)].
 40 Sequence No. 7 TCAACAAGCT GGAACGGACG (SEQ ID NO: 9)
 Sequence No. 8 CGCCATTAA CGGCCTTGAG (SEQ ID NO: 10)
 Sequence No. 9 CCGTCTGGAA CCACGCCGA T (SEQ ID NO: 11)
 Sequence No. 10 ATCGGGCGTG ATTCCAGACG G (SEQ ID NO: 12)

45 Sequence Nos. 7 and 8 have homologous sequences upstream and downstream from the pheA gene, respectively. Sequence Nos. 9 and 10 are complementary to each other and have almost perfect homology to the sequence around the 330 serine residue (³³⁰Ser), except that one base pair is different, i.e., T (thymine base) is substituted with C (cytosine base). CM-PD of Escherichia coli K-12 has high homology to PD of Brevibacterium lactofermentum. In particular, the 330 serine residue from the N-terminal (³³⁰Ser) of the CM-PD of Escherichia coli K-12 corresponds to the 235 serine residue (²³⁵Ser) of Brevibacterium lactofermentum PD. Sequence Nos 9 and 10 are synthesized in such a manner that the 330 serine residue becomes a proline residue.

50 Next, using 1 µg of the chromosomal DNA and either 300 ng of each of the primers of Sequence Nos. 7 and 10, or 300 ng of each of the primers of Sequence Nos. 8 and 9, PCR was carried out to obtain DNA fragments of 1.3 Kbp and 0.5 Kbp, respectively. The PCR temperature cycle of reaction at 94°C for one minute, at 50°C for 2 minutes and at 72°C for 3 minutes was repeated for 20 cycles using a continuous replication reaction device (Thermal Cycler, manufactured by Perkin Elmer Cetus Co.), according to the method of Erlich et al. [PCR Technology, Stockton Press (1989)]. These DNA fragments were subjected to agarose gel electrophoresis and recovered using a standard DNA recovery kit (Gene Clean, manufactured by Funakoshi Co.).

Separately, using these fragments and the primers of Sequence Nos. 7 and 8, PCR reaction was further carried out to obtain a DNA fragment of 1.8 Kbp. After the 1.8 Kbp fragment was digested with BamH1 and PstI, a DNA fragment of 1.7 Kbp was recovered by agarose electrophoresis. Subsequently, the 1.7 Kbp fragment was ligated with the BamH1 and PstI digestion product of plasmid pHSG398 (manufactured by Takara Shuzo) using T4 DNA ligase. The ligation product was used to transfet Escherichia coli KA197 strain (pheA). Among the strains resistant to chloramphenicol, the strain in which phenylalanine auxotrophy disappeared was selected, and a plasmid was recovered. The plasmid was named pPHAB. Its nucleotide sequence was determined. This plasmid bears the mutant CM-PD enzyme gene in which the 330 serine residue was substituted with a proline residue.

Also using the same methods mentioned above, 330 serine residue from the N-terminal (³³⁰Ser) of the CM-PD of Escherichia coli K-12 was substituted with an aspartic acid residue. Sequence Nos. 11 and 12 were synthesized in such a manner that the 330 serine residue became an aspartic acid residue.

Nos. 11 CCGTCTGGAA GACCGCCCCGA T
 Nos. 12 ATCGGGCGGT CTTCCAGACG G

In this way, the plasmid pPHAD, which bears the mutant CM-PD enzyme gene in which the 330 serine residue was substituted with an aspartic acid, was obtained.

Also using the same methods mentioned above, amino acid residues downstream from ³³⁰Ser of the CM-PD of Escherichia coli K-12 were deleted. Sequence Nos. 13 and 14 were synthesized in such a manner that the codon of the 330 serine residue became termination codon.

Nos. 13 CCGTCTGGAA TGACGCCCGA T
 Nos. 14 ATCGGGCGTC ATTCCAGACG G

In this way, the plasmid pPHATerm, which bears the mutant CM-PD enzyme gene in which the amino acid residues downstream from the 330 serine residue were deleted, was obtained.

(3) Construction of a tyrA gene-defected W3110 strain of Escherichia coli K-12

Escherichia coli W3110 strain (acquired from National Institute of Heredity) was spread on a plate medium containing streptomycin to obtain a streptomycin-resistant strain. Next, this strain was cultured in a culture medium in combination with Escherichia coli K-12 ME8424 strain (HfrPO45, thi, relA1, tyrA: Tn10, ung-1, nadB) (acquired from National Institute of Heredity), and the medium was allowed to stand at 37°C for 15 minutes to perform conjugation transfer. Then the medium was applied to a plate medium containing streptomycin, tetracycline and L-tyrosine. The formed colony, i.e., Escherichia coli W3110 (tyrA) strain, was collected.

The plasmid pPHAB, pPHAD, and pPHATerm obtained in Example (2)-2 above were used to transform competent cells of the E. coli W3110 (tyrA) strain. The transformant Escherichia coli K-12 [W3110 (tyrA)/pPHAB] Escherichia coli K-12 [W3110 (tyrA)/pPHAD], and Escherichia coli K-12 [W3110 (tyrA)/pPHATerm] were deposited in the Fermentation Research Institute of the Agency of industrial Science & Technology of Japan. The deposit numbers are FERM BP-3566, FERM BP-12659, and FERM BP-12662, respectively.

(4) Measurement of PD enzyme activity

Escherichia coli K-12 W3110 (tyrA)/(pPHAB) strain was cultured at 37°C for 15 hours in L medium and the cells were collected by centrifugation. Then, the cells were washed twice with physiological saline, and suspended in 250 mM Tris-hydrochloride buffer (pH 7.5) containing 0.5 mM dithiothreitol under ice cooling. By ultrasonication (20 KHz) for 30 seconds four times, the crude enzyme solution was prepared.

The PD enzyme activity was determined in a conventional manner [Cotton, R.G.H. and Gibson, F., Meth. in Enzy-mol., 17, 564 (1970)]. Using the crude enzyme, the enzymatic reaction was carried out at 37°C for 10 minutes in the presence of 50 mM Tris-hydrochloride buffer (pH 8.2) containing 1 mM barium prephenate and 0.5 mM L-tyrosine. Aqueous sodium hydroxide (1 N) was added to terminate the reaction, and the formed phenylpyruvic acid was measured at an extinction wavelength of 320 nm.

Quantitative determination of protein was made using the Protein Assay Kit (manufactured by Bio Rad Co.), according to the protocol of the manufacturer.

The results presented in Fig. 4 show that the enzyme reaction in strains transformed with the wild CM-PD gene was strongly inhibited in the presence of 0.5 mM L-phenylalanine, whereas strains transformed with a mutant CM-PD gene exhibited almost no inhibition, even in the presence of 5 mM L-phenylalanine.

Further, in the case of the plasmid bearing the wild type enzyme gene, in the absence of L-phenylalanine, the enzyme activity was only 3.5×10^2 U/mg protein. In the case of the mutant CM-PD gene, the activity was 1.5×10^4 U/mg

protein. The results show that not only is expression of the mutant type CM-PD enzyme gene released from feedback inhibition by L-phenylalanine in transformants containing the mutant gene, but surprisingly, the amount of enzyme and/or enzyme activity can be increased by roughly two orders of magnitude.

5 By using the same method above, the PD enzyme activities of *Escherichia coli* W3110 (*tyrA*)/(pPHAD) and *Escherichia coli* W3110 (*tyrA*)/(pPHATerm) were determined. As the result, the PD enzymes of the both strains were found to be released from feedback inhibition by L-phenylalanine.

Example 3: Production of L-phenylalanine by fermentation

10 (1) Construction of *Escherichia coli* K-12 bearing a CM-PD gene alone and, in combination with a DS gene, in which the feedback inhibition is released

From pTS-aroG4 bearing the feedback inhibition-released DS gene obtained in Example 1, the aroG4 portion was excised with restriction enzymes EcoRI and HindIII. The fragment was inserted into the cleavage site of pBR322 with 15 EcoRI and HindIII to obtain plasmid pBR-aroG4 (having an ampicillin-resistant marker).

In a separate procedure, pPHAB bearing the feedback inhibition-released CM-PD gene obtained in Example 2 was digested with restriction enzymes BamHI and HindIII to excise the fragment containing the CM-PD gene. This fragment was inserted into the cleavage site of pACYC184 with BamHI and HindIII to construct plasmid pACMAB (selection 20 marker was chloramphenicol resistance). The pACMAB plasmid was used to transform competent cells of *Escherichia coli* K-12 W3110 (*tyrA*), yielding transformant W3110 (*tyrA*)/pACMAB.

Furthermore, the two plasmids pACMAB and pBR-aroG4 were used to transform W3110 (*tyrA*) yielding transformant W3110 (*tyrA*)/pBR-aroG4, pACMAB. The transformant W3110 (*tyrA*)/pBR-aroG4, pACMAB was named AJ 12604 under the deposit number FERM BP-3579.

25 (3) Production of L-phenylalanine

The transformant AJ 12604, W3110 (*tyrA*)/pACMAB, W3110 (*tyrA*)/pPHAD, W3110 (*tyrA*)/pPHATerm, and W3110 (*tyrA*) described above were cultured at 37°C for 24 hours in L-phenylalanine-producing medium (containing 20 g of glucose, 29.4 g of disodium hydrogen phosphate, 6 g of potassium dihydrogen phosphate, 1 g of sodium chloride, 2 g of ammonium chloride, 10 g of sodium citrate, 0.4 g of sodium glutamate, 3 g of magnesium sulfate heptahydrate, 0.23 g of calcium chloride and 2 mg of thiamine hydrochloride in 1 liter of water). The results are shown in Table 2. Quantitative assay was performed by high performance liquid chromatography. An outstanding improvement in the fermentative production of L-phenylalanine using the AJ 12604 strain was obtained.

Table 2

| Strain | Amount of L-phenylalanine |
|--------------------------------|---------------------------|
| W3110(<i>tyrA</i>) | 0.1 |
| W3110 (<i>tyrA</i>)/pACMAB | 0.5 |
| W3110 (<i>tyrA</i>)/pPHAD | 0.5 |
| W3110 (<i>tyrA</i>)/pPHATerm | 0.5 |
| AJ 12604 | 3.8 |

50 Example 4: Production of L-tryptophan by fermentation

(1) Construction of a plasmid bearing feedback inhibition-released DS

55 Plasmid pACYC177 (acquired from National Institute of Heredity; ampicillin resistance, 3.6 Kbp) was digested with restriction enzyme XbaI. After the digestion site was made blunt ended by Klenow treatment, an EcoRI linker was ligated therewith using T4 DNA ligase to obtain the plasmid pACYC177E, in which the XbaI site became EcoRI. Next, the plasmid pTS-aroG4 described in Example 1-(2) and 1-(3) above was digested with restriction enzymes EcoRI and HindIII to obtain the fragment containing aroG4. This fragment was ligated with the EcoRI- and HindIII-digested

pACYC177E using T4 DNA ligase. Competent cells of the AB3257 strain (described in Example 1) was transformed with the reaction mixture. Among the ampicillin-resistant strains grown, a strain in which auxotrophy for each of L-tyrosine, L-phenylalanine and L-tryptophane disappeared was selected, and a plasmid was extracted. Thus, plasmid pACEG4 (5.1 Kbp) was obtained. An EcoRI-EcoRI fragment containing a gene conferring kanamycin resistance (Kanamycin Gene Block; 1.3 Kbp, manufactured by Pharmacia Fine Chemicals) was ligated with the plasmid pACEG4 at the EcoRI site using T4 DNA ligase, thus yielding the plasmid pACKG4 (resistant to ampicillin and kanamycin, 6.4 KbP). The procedure of the construction of pACKG4 is outlined in Fig. 5.

10 (2) Construction of Escherichia coli K-12 bearing a feedback inhibition-released DS gene and a tryptophan operon

15 Competent cells of Escherichia coli K-12 AGX6aroP strain (described in U.S. Patent No. 4,371,614, incorporated herein by reference; deposit number: NRRL B-12264) bearing the plasmid pGX50 harboring a tryptophan operon was transformed with the pACKG4 plasmid described above to obtain Escherichia coli AGX6aroP/pGX50,pACKG4. The genotype of Escherichia coli AGX6aroP strain is tna, trpR+, aroP.

15 (3) Production of L-tryptophan

20 The transformant Escherichia coli AGX6aroP/pGX50,pACKG4 and AGX6aroP/pGX50 described above was cultured at 30°C for 72 hours in L-tryptophan-producing medium (containing 40 g of glucose, 15 g of ammonium sulfate, 1 g of potassium monohydrogen phosphate, 1 g of magnesium sulfate heptahydrate, 0.01 g of ferrous sulfate heptahydrate, 0.01 g of manganese chloride tetrahydrate, 2 g of yeast extract and 40 g of calcium carbonate in 1 liter of water, pH 7). The results are shown in Table 3. Quantitative assay of L-tryptophan was performed by high performance liquid chromatography. An outstanding improvement in the fermentative production of L-tryptophan using the AGX-aroP/pGX50,pACKG strain was obtained.

25

Table 3

| Strain | Amount of L-tryptophan Produced (g/l) |
|-----------------------|---------------------------------------|
| AGX6aroP/pGX50 | 0.15 |
| AGX6aroP/pGX50,pACKG4 | 0.45 |

35

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

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SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT: Ajinomoto Co., Ltd.

10 (ii) TITLE OF INVENTION: GENES ENCODING FEEDBACK
INHIBITION-RELEASED ENZYMES, PLASMIDS CONTAINING THE
GENES, AND MICROORGANISMS TRANSFORMED WITH THE PLASMIDS
USEFUL IN PROCESSES FOR PREPARING AROMATIC AMINO ACIDS BY
FERMENTATION

15 (iii) NUMBER OF SEQUENCES: 12

20 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

30

35

40

45

50

55

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli
10 (B) STRAIN: K-12 MC1061

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAACCAAGT AAAGCCAACA

20

15

(2) INFORMATION FOR SEQ ID NO:2:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli
30 (B) STRAIN: K-12 MC1061

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCCACITTCAG CAACCAGTTTC

20

35

(2) INFORMATION FOR SEQ ID NO:3:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

50

55

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli
(B) STRAIN: K-12 MC1061

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTATTTACCC CGTTATTGTC

10

20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: DNA (genomic)

20

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli
(B) STRAIN: K-12 MC1061

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACTCCGCCGG AAGTGACTAA

30

20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 948 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: DNA (genomic)

40

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Brevibacterium lactofermentum
(B) STRAIN: AJ 12125

45

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..945

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | |
|---|-----|
| ATG ACC CAC CCA CCA ATT GTT GTG GCC TAT TTG GGG CCT GCC GGA ACC Met Ser Asp Ala Pro Ile Val Val Ala Tyr Leu Gly Pro Ala Gly Thr 1 5 10 15 | 48 |
| TTC ACC GAA GAA GCC CTC TAC AAA TTT GCC GAC GCC GGC GTA TTC GGC Phe Thr Glu Glu Ala Leu Tyr Lys Phe Ala Asp Ala Gly Val Phe Gly 20 25 30 | 96 |
| GAC GGT GAG ATC GAG CAG CTA CCA GCC AAA TCG CCA CAA GAA GCT GTC Asp Gly Glu Ile Glu Gln Leu Pro Ala Lys Ser Pro Gln Glu Ala Val 35 40 45 | 144 |
| } GAC GCG GTC CGC CAC GGC ACC GCC CAG TTC GCG GTG GTC GCC ATC GAA Asp Ala Val Arg His Gly Thr Ala Gln Phe Ala Val Val Ala Ile Glu 50 55 60 | 192 |
| AAC TTC GTC GAC GGC CCC GTC ACC CCC ACC TTC GAC GCC CTT GAC CAG Asn Phe Val Asp Gly Pro Val Thr Pro Thr Phe Asp Ala Leu Asp Gln 65 70 75 80 | 240 |
| GGC TCC AAC GTG CAA ATC ATC GCC GAA GAA GAA CTC GAT ATT GCC TTT Gly Ser Asn Val Gln Ile Ile Ala Glu Glu Glu Leu Asp Ile Ala Phe 85 90 95 | 288 |
| TCC ATC ATG GTC CGG CCA GGG ACT TCG CTT GCC GAC GTC AAA ACC CTC Ser Ile Met Val Arg Pro Gly Thr Ser Leu Ala Asp Val Lys Thr Leu 100 105 110 | 336 |
| GCC ACC CAC CCG GTT GGG TAC CAA CAA GTG AAA AAC TGG ATG GCA ACC Ala Thr His Pro Val Gly Tyr Gln Gln Val Lys Asn Trp Met Ala Thr 115 120 125 | 384 |
| ACC ATT CCG GAC GCC ATG TAT CTT TCA GCA AGC TCC AAC GGC GCC GGC Thr Ile Pro Asp Ala Met Tyr Leu Ser Ala Ser Ser Asn Gly Ala Gly 130 135 140 | 432 |
| GCA CAA ATG GTT GCC GAA GGA ACC GCC GAC GCA GCC GCA GCG CCC TCC Ala Gln Met Val Ala Glu Gly Thr Ala Asp Ala Ala Ala Pro Ser 145 150 155 160 | 480 |
| CGC GCA GCC GAA CTC TTC GGA CTG GAA CGC CTT GTT GAT GAT GTC GCC Arg Ala Ala Glu Leu Phe Gly Leu Glu Arg Leu Val Asp Asp Val Ala 165 170 175 | 528 |
| GAC GTC CGC GGC GCC CGC ACC CGC TTC GTT GCA GTC CAA GCC CAA GCA Asp Val Arg Gly Ala Arg Thr Arg Phe Val Ala Val Gln Ala Gln Ala 180 185 190 | 576 |
| GCC GTT TCC GAA CCG ACC GGC CAC GAC CGC ACC TCC GTC ATT TTC TCC Ala Val Ser Glu Pro Thr Gly His Asp Arg Thr Ser Val Ile Phe Ser 195 200 205 | 624 |

| | | |
|----|---|-----|
| 5 | CTA CCG AAT GTG CCA GGC AGC CTC GTG CGC GCC CTC AAC GAA TTC GCC Leu Pro Asn Val Pro Gly Ser Leu Val Arg Ala Leu Asn Glu Phe Ala 210 215 220 | 672 |
| | ATC CGT GGC GTC GAC CTC ACC CGC ATC GAA TCC CGC CCC ACC CGC AAA Ile Arg Gly Val Asp Leu Thr Arg Ile Glu Ser Arg Pro Thr Arg Lys 225 230 235 240 | 720 |
| 10 | GTC TTC GGA ACC TAC CGC TTC CAC CTG GAC ATA TCC GGA CAT ATC CGC Val Phe Gly Thr Tyr Arg Phe His Leu Asp Ile Ser Gly His Ile Arg 245 250 255 | 768 |
| | GAC ATC CCC GTC GCC GAA GCC CTC CGC GCA CTC CAC CTC CAA GCC GAA Asp Ile Pro Val Ala Glu Ala Leu Arg Ala Leu His Leu Gln Ala Glu 260 265 270 | 816 |
| 15 | GAA CTC GTA TTC GTC GGT TCC TGG CCC TCC AAC CGT GCA GAA GAC AGC Glu Leu Val Phe Val Gly Ser Trp Pro Ser Asn Arg Ala Glu Asp Ser 275 280 285 | 864 |
| | ACG CCC CAA ACC GAC CAA CTA GCT AAC GTC CAC AAG GCG GAC GAA TGG Thr Pro Gln Thr Asp Gln Leu Ala Asn Val His Lys Ala Asp Glu Trp 290 295 300 | 912 |
| | GTT CGC GCA GCA AGC GAA GGA AGG AAA CTT AAC TAG Val Arg Ala Ala Ser Glu Gly Arg Lys Leu Asn 305 310 315 | 948 |
| 25 | (2) INFORMATION FOR SEQ ID NO:6: | |
| | (i) SEQUENCE CHARACTERISTICS: | |
| 30 | (A) LENGTH: 315 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear | |
| | (ii) MOLECULE TYPE: protein | |
| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6: | |
| 35 | Met Ser Asp Ala Pro Ile Val Val Ala Tyr Leu Gly Pro Ala Gly Thr 1 5 10 15 | |
| | Phe Thr Glu Glu Ala Leu Tyr Lys Phe Ala Asp Ala Gly Val Phe Gly 20 25 30 | |
| 40 | Asp Gly Glu Ile Glu Gln Leu Pro Ala Lys Ser Pro Gln Glu Ala Val 35 40 45 | |
| | Asp Ala Val Arg His Gly Thr Ala Gln Phe Ala Val Val Ala Ile Glu 50 55 60 | |
| 45 | | |

Asn Phe Val Asp Gly Pro Val Thr Pro Thr Phe Asp Ala Leu Asp Gln
 65 70 75 80

5 Gly Ser Asn Val Gln Ile Ile Ala Glu Glu Glu Leu Asp Ile Ala Phe
 85 90 95

Ser Ile Met Val Arg Pro Gly Thr Ser Leu Ala Asp Val Lys Thr Leu
 100 105 110

10 Ala Thr His Pro Val Gly Tyr Gln Gln Val Lys Asn Trp Met Ala Thr
 115 120 125

Thr Ile Pro Asp Ala Met Tyr Leu Ser Ala Ser Ser Asn Gly Ala Gly
 130 135 140

15 Ala Gln Met Val Ala Glu Gly Thr Ala Asp Ala Ala Ala Pro Ser
 145 150 155 160

20 Arg Ala Ala Glu Leu Phe Gly Leu Glu Arg Leu Val Asp Asp Val Ala
 165 170 175

Asp Val Arg Gly Ala Arg Thr Arg Phe Val Ala Val Gln Ala Gln Ala
 180 185 190

25 Ala Val Ser Glu Pro Thr Gly His Asp Arg Thr Ser Val Ile Phe Ser
 195 200 205

30 Leu Pro Asn Val Pro Gly Ser Leu Val Arg Ala Leu Asn Glu Phe Ala
 210 215 220

Ile Arg Gly Val Asp Leu Thr Arg Ile Glu Ser Arg Pro Thr Arg Lys
 225 230 235 240

35 Val Phe Gly Thr Tyr Arg Phe His Leu Asp Ile Ser Gly His Ile Arg
 245 250 255

Asp Ile Pro Val Ala Glu Ala Leu Arg Ala Leu His Leu Gln Ala Glu
 260 265 270

40 Glu Leu Val Phe Val Gly Ser Trp Pro Ser Asn Arg Ala Glu Asp Ser
 275 280 285

Thr Pro Gln Thr Asp Gln Leu Ala Asn Val His Lys Ala Asp Glu Trp
 290 295 300

45 Val Arg Ala Ala Ser Glu Gly Arg Lys Leu Asn
 305 310 315

50 (2). INFORMATION FOR SEQ ID NO:7:

(1) SEQUENCE CHARACTERISTICS:

5

- (A) LENGTH: 948 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Brevibacterium lactofermentum*
 - (B) STRAIN: AJ 12259

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

| | | |
|----|--|-----|
| | ATGAGCGACGCACCAATTGTGTGGCTATTGGGGCTGCCGGAACCTTCACCGAAGAA | 60 |
| | GCCCTCTACAAATTGCGCACGCCGGCGTATTCGGCGACGGTGAGATCGAGCAGCTACCA | 120 |
| 20 | GCCAAATCGCCACAAGAAGCTGTCGACGCCGGCACGGCACGCCAGTTCGCGGTG | 180 |
| | GTGCCCATCGAAAACCTTCGTCGACGGCCCCGTACCCCCACCTTCGACGCCCTTGACCAG | 240 |
| | GGCTCCAACGTGCAAATCATCGCCGAAGAAGAAACTCGATATTGCTTTCCATCATGGTC | 300 |
| 25 | CGGCCAGGGACTTCGCTTGCCGACGTCAAACCTCGCACCCACCCGGTTGGGTACCAA | 360 |
| | CAAGTAAAAACTGGATGGCAACCACCATCCGGACGCCATGTATCTTCAGCAAGCTCC | 420 |
| | AACGGCGCCGGCGACAAATGGTTGCCGAAGGAACCGCCACGCAGCCGAGCGCCCTCC | 480 |
| 30 | CGCGCAGCCGAACTCTCGGACTGGAACGCCCTGGATGATGTCGCCACGTCCGGC | 540 |
| | GCCCCCACCCGCTTCGTTGCAGTCCAAGCCCAAGCAGCCGTTCCGAACCGACCGCCAC | 600 |
| | GACCGCACCTCCGTCACTTCCTACCGAATGTGCCAGGCAGCCTCGTCCGGCCCTC | 660 |
| 35 | AACGAATTGCCATCCGTGGCGTGCACCTCACCCGATCGAATCCGCCAACCGCAA | 720 |
| | GTCTTCGGAACCTACCGCTTCCACCTGGACATATCCGGACATATCCGCACATCCCCGTC | 780 |
| | GCCGAAGCCCTCCGGCACTCCACCTCCAAGCGAAGAACTCGTATTGTCGGTTCCGG | 840 |
| 40 | CCCTCCAACCGTGAGAAGACAGCACGCCAACCGACCAACTAGCTAACGTACACAAG | 900 |
| | GCGGACGAATGGGTCCGGCAGCAAGCGAAGGAAGGAAACTTAACCTAG | 948 |

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(2) INFORMATION FOR SEQ ID NO:8:

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(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 315 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

| | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Met | Ser | Asp | Ala | Pro | Ile | Val | Val | Ala | Tyr | Leu | Gly | Pro | Ala | Gly | Thr |
| 1 | | | | | | 5 | | | | | 10 | | | | | 15 |
| 15 | Phe | Thr | Glu | Glu | Ala | Leu | Tyr | Lys | Phe | Ala | Asp | Ala | Gly | Val | Phe | Gly |
| | | | | | | 20 | | 25 | | | | | | 30 | | |
| 20 | Asp | Gly | Glu | Ile | Glu | Gln | Leu | Pro | Ala | Lys | Ser | Pro | Gln | Glu | Ala | Val |
| | | | | | 35 | | | 40 | | | | | | 45 | | |
| 25 | Asp | Ala | Val | Arg | His | Gly | Thr | Ala | Gln | Phe | Ala | Val | Val | Ala | Ile | Glu |
| | | | | | 50 | | | 55 | | | | | 60 | | | |
| 30 | Asn | Phe | Val | Asp | Gly | Pro | Val | Thr | Pro | Thr | Phe | Asp | Ala | Leu | Asp | Gln |
| | | 65 | | | | | 70 | | 75 | | | | | 80 | | |
| 35 | Gly | Ser | Asn | Val | Gln | Ile | Ile | Ala | Glu | Glu | Leu | Asp | Ile | Ala | Phe | |
| | | | | | 85 | | | 90 | | | | | 95 | | | |
| 40 | Ser | Ile | Met | Val | Arg | Pro | Gly | Thr | Ser | Leu | Ala | Asp | Val | Lys | Thr | Leu |
| | | 100 | | | | | | 105 | | | | | 110 | | | |
| 45 | Ala | Thr | His | Pro | Val | Gly | Tyr | Gln | Gln | Val | Lys | Asn | Trp | Met | Ala | Thr |
| | | | | | 115 | | | 120 | | | | | 125 | | | |
| 50 | Thr | Ile | Pro | Asp | Ala | Met | Tyr | Leu | Ser | Ala | Ser | Ser | Asn | Gly | Ala | Gly |
| | | | | | 130 | | | 135 | | | | | 140 | | | |
| 55 | Ala | Gln | Met | Val | Ala | Glu | Gly | Thr | Ala | Asp | Ala | Ala | Ala | Pro | Ser | |
| | | | | | 145 | | | 150 | | | | 155 | | 160 | | |
| 60 | Arg | Ala | Ala | Glu | Leu | Phe | Gly | Leu | Glu | Arg | Leu | Val | Asp | Asp | Val | Ala |
| | | | | | 165 | | | 170 | | | | | 175 | | | |
| 65 | Asp | Val | Arg | Gly | Ala | Arg | Thr | Arg | Phe | Val | Ala | Val | Gln | Ala | Gln | Ala |
| | | | | | 180 | | | 185 | | | | | 190 | | | |
| 70 | Ala | Val | Ser | Glu | Pro | Thr | Gly | His | Asp | Arg | Thr | Ser | Val | Ile | Phe | Ser |
| | | | | | 195 | | | 200 | | | | | 205 | | | |
| 75 | Leu | Pro | Asn | Val | Pro | Gly | Ser | Leu | Val | Arg | Ala | Leu | Asn | Glu | Phe | Ala |
| | | | | | 210 | | | 215 | | | | | 220 | | | |
| 80 | Ile | Arg | Gly | Val | Asp | Leu | Thr | Arg | Ile | Glu | Pro | Arg | Pro | Thr | Arg | Lys |
| | | | | | 225 | | | 230 | | | | | 235 | | 240 | |

Val Phe Gly Thr Tyr Arg Phe His Leu Asp Ile Ser Gly His Ile Arg
 245 250 255
 5 Asp Ile Pro Val Ala Glu Ala Leu Arg Ala Leu His Leu Gln Ala Glu
 260 265 270
 Glu Leu Val Phe Val Gly Ser Trp Pro Ser Asn Arg Ala Glu Asp Ser
 275 280 285
 10 Thr Pro Gln Thr Asp Gln Leu Ala Asn Val His Lys Ala Asp Glu Trp
 290 295 300
 Val Arg Ala Ala Ser Glu Gly Arg Lys Leu Asn
 305 310 315

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(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Escherichia coli
 - (B) STRAIN: K-12 RR1

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCAACAAAGCT GGAACGGACG

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(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:

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(A) ORGANISM: *Escherichia coli*
(B) STRAIN: K-12 RR1

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCCGATTTA CGGCCTTGAG

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10 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20 (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGTCTGGAA CCACGCCCGA T

21

25 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30 (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

40 ATCGGGCGTG ATTCCAGACG G

21

45 Claims

1. A DNA fragment containing a mutated aroF gene encoding 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase, in which the amino acid residues in positions 147 and/or 181 are substituted or one or more amino acid residue(s) are deleted or added, and which is released from feedback inhibition.
- 50 2. A DNA fragment containing a mutated aroG gene encoding 3-deoxy-D-arabinoheptulonic acid-7-phosphate synthase, in which one or more amino acid residues in positions 146, 147, 150, 157, 202, 219, and 332 are substituted or one or more amino acid residue(s) are deleted or added, and which is released from feedback inhibition.
- 55 3. A DNA fragment according to claim 1, in which the aspartate residue 147 is substituted by an asparagine and the serine residue 181 is substituted by a phenylalanine.
4. A DNA fragment according to claim 2, in which the substituent of the aspartate residue 146 is an asparagine residue, the substituent of the methionine residue 147 is an isoleucine residue, the substituent of the proline residue

150 is a leucine residue, the substituent of the methionine residue 157 is an isoleucine residue, the substituent of the alanine residue 202 is a threonine residue, the substituent of the alanine residue 219 is a threonine residue, and the substituent of the glutamate residue in position 332 is a lysine residue.

- 5 5. A vector, in which the DNA fragment according to any of claims 1 to 4 is operably linked to regulatory DNA effecting expression of said protein encoding DNA.
6. A microorganism comprising a vector according to claim 5.
- 10 7. A microorganism, in which the DNA fragment according to any of claims 1 to 4 is integrated into the chromosome and is operably linked to regulatory DNA effecting expression of said protein encoding DNA.
- 15 8. A microorganism according to claim 6 or 7, which additionally comprises a mutated gene encoding chorismate mutase-prephenate-dehydratase from *Escherichia coli* or prephenate-dehydratase from a *Coryneform* bacterium, wherein one or two amino acid residue(s) are substituted or one or more amino acid residue(s) are deleted.
- 20 9. A microorganism according to claim 8, wherein the serine residue in position 330 of chorismate mutase-prephenate-dehydratase is substituted or amino acid residues downstream from the 330 serine residue are deleted or wherein the serine residue in position 235 of prephenate-dehydratase is substituted.
- 25 10. A microorganism according to claim 9, wherein said serine residues are substituted by a proline residue or an aspartate residue.
11. A process for preparing an aromatic amino acid which comprises culturing a microorganism claimed in any of claims 6 to 10 in a medium and isolating the aromatic amino acid produced.
12. A process for preparing an amino acid as claimed in claim 11, wherein said aromatic amino acid is L-phenylalanine or L-tryptophan.

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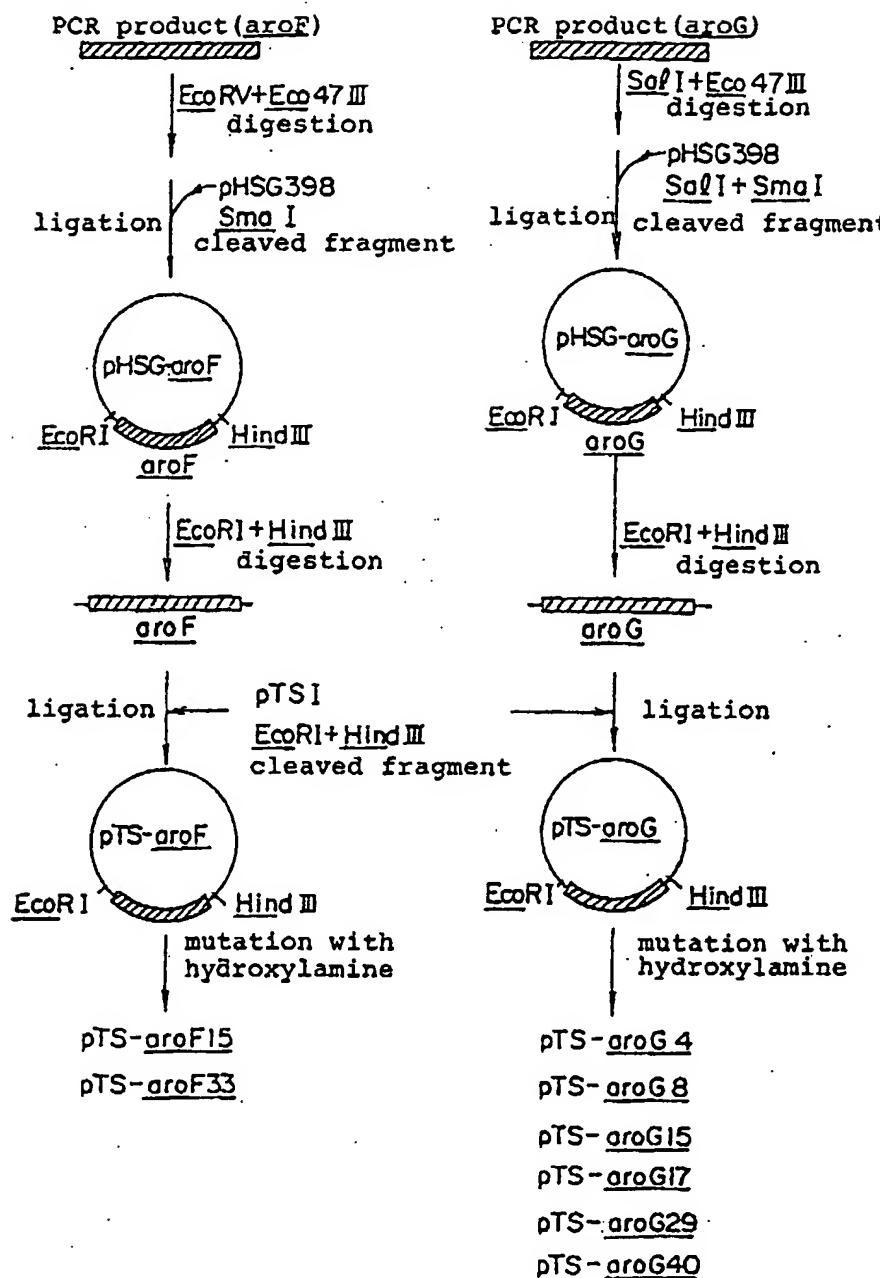
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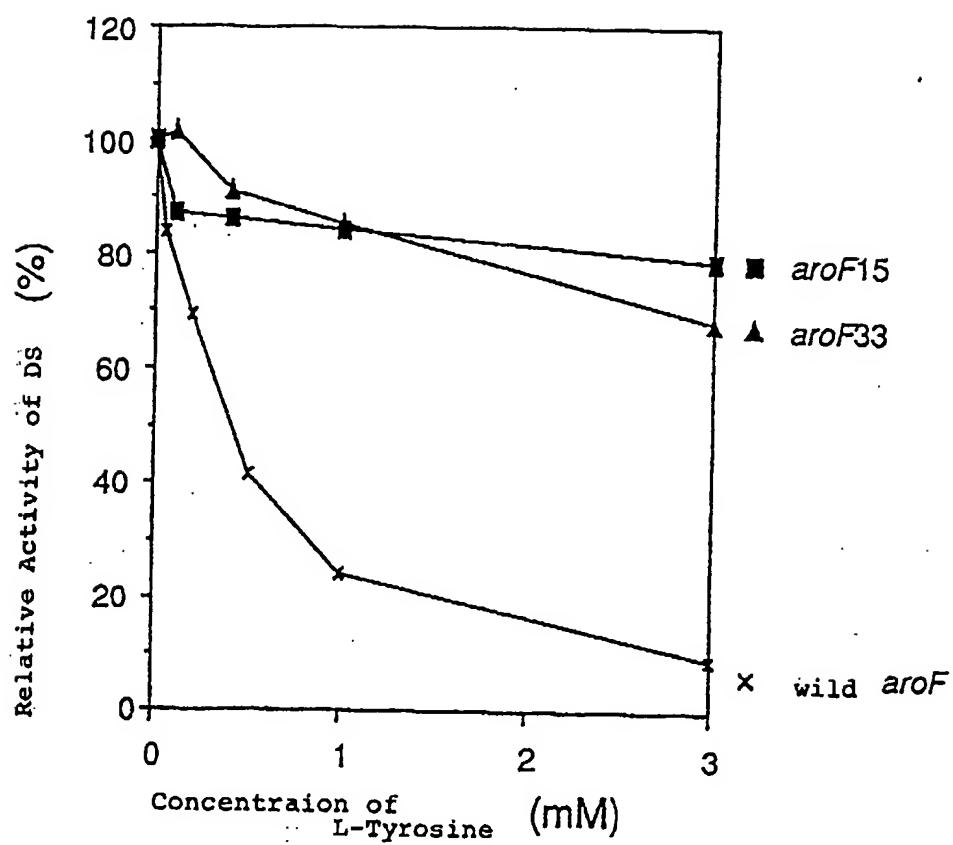
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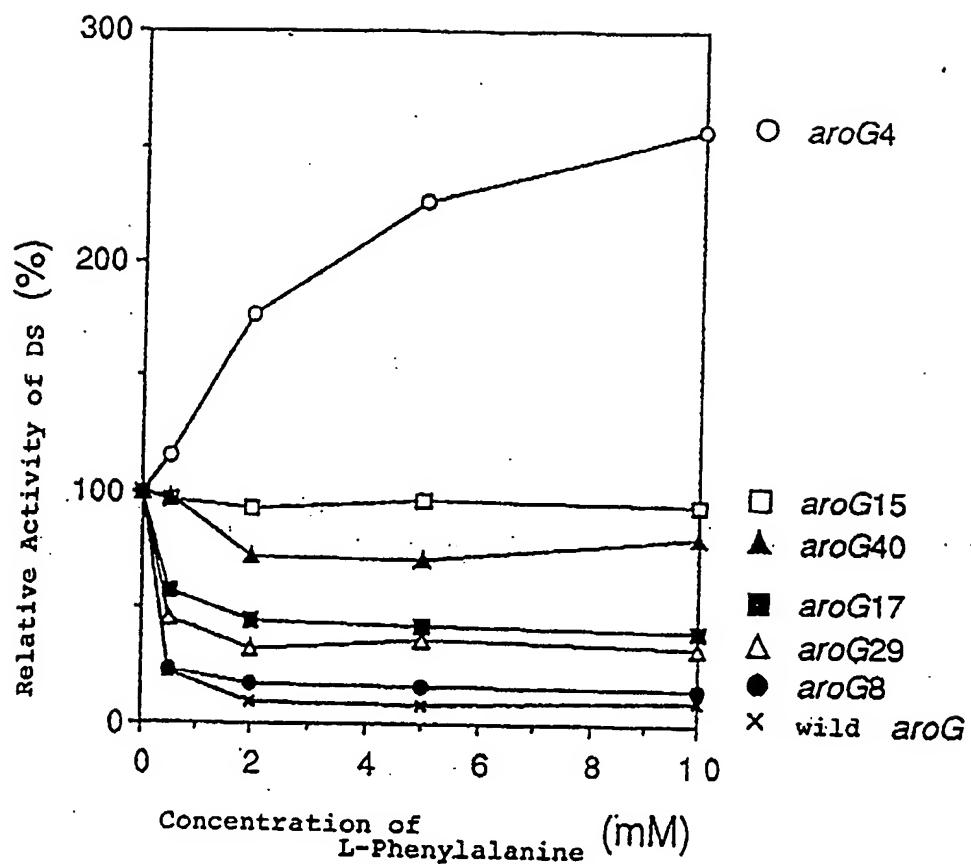
(FIG.1)



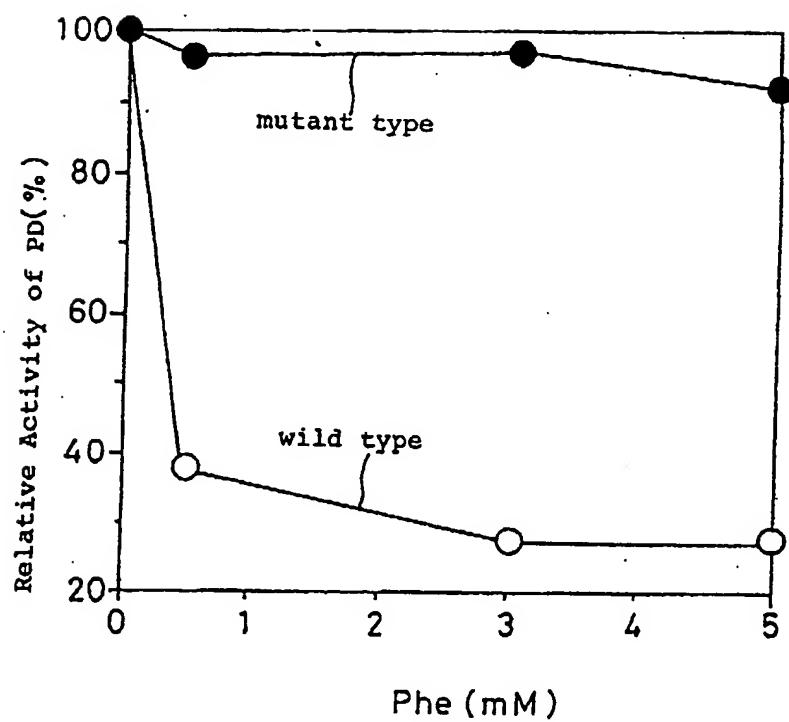
(FIG. 2)



(FIG. 3)



(FIG. 4)



(FIG. 5)

